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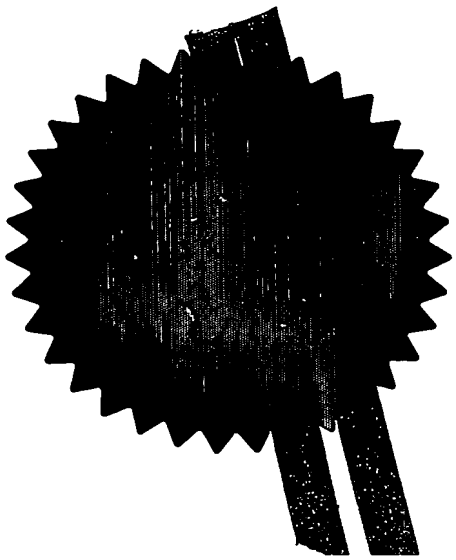

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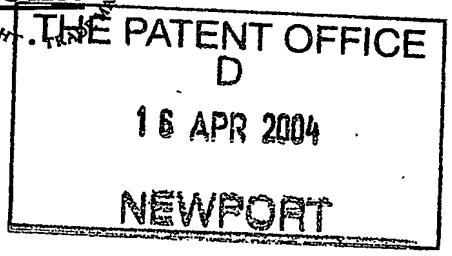

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Patents ADP number *(if you know it)*

6594709001

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4. Title of the invention — Use

5. Name of your agent *(if you have one)* — Harrison Goddard Foote

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Signature(s) Harrison Goddard Foote Date 15/4/04

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15 April 2004

Michelle O'Neill 01904 732 120

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USE

This invention relates to the use of the poly(ADP-ribose) polymerase (PARP) inhibitor, AG14361, in the treatment of certain forms of cancer in particular breast cancer.

Homologous recombination (HR) has been shown to play an important role in repair of damage occurring at DNA replication forks in mammalian cells (2). Thus, cells deficient in HR show retarded growth and exhibit higher level of genetic instability. It is believed that genetic instability due to loss of HR repair in human cancers significantly contributes to the development of cancer in these cells (1).

Post transcriptional modification of nuclear proteins by poly(ADP-ribosyl)ation (PARP) in response to DNA strand breaks plays an important role in DNA repair, regulation of apoptosis, and maintenance of genomic stability.

Poly(ADP-ribose) Polymerase (PARP-1) is an abundant nuclear protein in mammalian cells that catalyses the formation of poly(ADP-ribose) (PAR) polymers using NAD^+ as substrate. Upon DNA damage, PARP-1 binds rapidly to a DNA single-strand break (SSB) and catalyses the addition of negatively charged PAR chains to itself (automodification) and other proteins (see (3, 4) for reviews). The binding of PARP-1 to SSBs is believed to protect DNA lesions from further processing until PARP-1 is dissociated from the break by the accumulated negative charge resulting from PAR polymers (5,6).

Although PARP-1 has been implicated in several nuclear processes, such as modulation of chromatin structure, DNA replication, DNA repair and transcription, PARP-1 knockout mice develop normally (7). Cells isolated from these mice exhibit a hyper recombination phenotype and genetic instability in the form of increased levels of SCE, micronuclei and tetraploidy (8-10). Genetic instability may also occur in these PARP-1 knockout mice through telomere shortening, increased frequency of chromosome fusion and aneuploidy (11), although all of these results could not be repeated in another set of PARP-1 knock-out mice (12). In the former mice knockout, PARP-1 null mutation rescue impaired V(D)J recombination in SCID mice (13).

These results support the view suggested by Lindahl and coworkers that PARP-1 has a protective role against recombination (5). They proposed that binding of PARP-1 to ssDNA breaks prevents the recombination machinery from recognizing and processing DNA lesions or, alternatively, that the negative charges accumulated following poly ADP-ribosylation repel adjacent recombinogenic DNA sequences. Only the latter model is consistent with inhibition of PARP-1 itself and expression of a dominant negative mutant PARP-1, inducing SCE, gene amplification and homologous recombination (HR [14-18]).

Studies based on treating cells with inhibitors of PARP-1 or cells derived from PARP-1 knockout mice indicate that the suppression of PARP-1 activity increases cell susceptibility to DNA damaging agents and inhibits strand break rejoining (3, 4, 8-11, 19, 20).

Inhibitors of PARP-1 activity have been used in combination with traditional anti-cancer agents such as radio therapy and chemotherapy (21). The inhibitors were used in combination with methylating agents, topoisomerase poisons and ionising radiations and were found to enhance the effectiveness of these forms of treatment. Such treatments, however, are known to cause damage and death to non cancerous or "healthy" cells and are associated with unpleasant side effects.

There is therefore a need for a treatment for cancer that is both effective and selective in the killing of cancer cells and which does not need to be administered in combination with radio or chemotherapy treatments.

The present inventors have surprisingly found that cells deficient in homologous recombination (HR) are hypersensitive to the PARP inhibitor, AG14361, as compared to wild type cells.

According to a first aspect of the invention there is provided the use of the PARP inhibitor, AG14361, in the manufacture of a medicament for the treatment of a disease or condition that is caused by a genetic defect in a gene that mediates homologous recombination.

The PARP inhibitor, AG14361, has been shown to inhibit the activity of PARP.

In a further aspect the invention provides a method of treatment of a disease or condition in a mammal, including human, which is caused by a genetic defect in a gene which mediates homologous recombination, which method comprises administering to the mammal a therapeutically effective amount of the PARP inhibitor, AG14361.

In a further preferred aspect, the use is in the treatment of cancer wherein the cancer is caused by a genetic defect in a gene which mediates homologous recombination.

Preferably the medicament is a pharmaceutical composition consisting of the PARP inhibitor in combination with a pharmaceutically acceptable carrier or diluent.

The specific sensitivity of HR defective tumours to PARP inhibition means that normally dividing cells in the patient will be unaffected by the treatment. Treatment of HR defective cancer cells using a PARP inhibitor also has the advantage that it does not need to be administered as a combination therapy along with conventional radio or chemotherapy treatments thereby avoiding the side effects associated with these conventional forms of treatment.

A defect in a gene that mediates homologous recombination may be due to a mutation in, the absence of, or defective expression of, a gene encoding a protein involved in HR.

In a further aspect, the invention further provides the use of the PARP inhibitor, AG14361, in the manufacture of a medicament for inducing apoptosis in HR defective cells.

In another aspect the invention provides a method of inducing apoptosis in HR defective cells in a mammal which method comprises administering to the mammal a therapeutically effective amount of the PARP inhibitor, AG14361. By causing apoptosis in HR defective cells it should be possible to reduce or halt the growth of a tumour in the mammal.

Preferably, the HR defective cells are cancer cells.

Cancer cells defective in HR may partially or totally deficient in HR. Preferably the cancer cells are totally deficient in HR.

The term "cancer" or "tumour" includes cancer of the lung, colon, pancreatic, gastric, ovarian, cervical, breast or prostate cancer. In a preferred aspect, the cancer is in a mammal, preferably human.

The cancer to be treated may be an inherited form of cancer wherein the patient to be treated has a familial predisposition to the cancer. Preferably, the cancer to be treated is gene-linked hereditary cancer. In a preferred embodiment of the invention the cancer is gene-linked hereditary breast cancer.

In a preferred aspect, the PARP inhibitor is useful in the treatment of cancer cells defective in the expression of a gene involved in HR. Genes with suggested function in HR include XRCC1, ADPRT (PARP-1), ADPRTL2 (PARP-2), CTPS, RPA, RPA1, RPA2, RPA3, XPD, ERCC1, XPF, MMS19, RAD51, RAD51B, RAD51C, RAD51D, DMC1, XRCC2, XRCC3, BRCA1, BRCA2, RAD52, RAD54, RAD50, MRE11, NBS1, WRN, BLM, Ku70, Ku80, ATM, ATR, chk1, chk2, FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, RAD1, RAD9 (see [2, 3, 5, 22-28] for reviews).

A gene involved in HR may be a tumour suppressor gene. The invention thus provides for the treatment of cancer cells defective in the expression of a tumour suppressor gene. Preferably, the tumour suppressor gene is BRCA1 or BRCA2.

Breast cancer is the most common cancer disease among women in the Western world today. Certain families have strong predisposition for breast cancer, which is often owing to an inherited mutation in one allele of either BRCA1 or BRCA2. However, these patients still maintain one functional allele. Thus, these patient develop normally and have no phenotypic consequence from this mutation. However, in one cell, the functional allele might be lost, making this cell cancerous and at the same time

deficient in homologous recombination (HR). This step is critical for the onset of a tumour [1].

In a preferred aspect, the invention provides the use of the PARP inhibitor, AG14361, in the manufacture of a medicament for the treatment of cancer cells defective in BRCA1 and/or BRCA2 expression.

The cancer cells to be treated may be partially or totally deficient in BRCA1 or BRCA2 expression. BRCA1 and BRCA2 mutations can be identified using multiplex PCR techniques, array techniques (29, 30) or using other screens known to the skilled person.

The PARP inhibitor formulated as a pharmaceutical composition may be administered in any effective, convenient manner effective for targeting cancer cells including, for instance, administration by oral, intravenous, intramuscular, intradermal, intranasal, topical routes among others. Carriers or diluents useful in the pharmaceutical composition may include, but are not limited to saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion. The inhibitor may be administered directly to a tumour or may be targeted to the tumour via systemic administration.

A therapeutically effective amount of the inhibitor is typically one which is sufficient to achieve the desired effect and may vary according to the nature and severity of the disease condition, and the potency of the inhibitor. It will be appreciated that different concentrations may be employed for prophylaxis than for treatment of an active disease.

For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01mg/kg to 10 mg/kg body weight, typically up to 0.1, 05, 1.0, 2.0 or 5.0 mg/kg body weight. Ultimately, however, the amount of inhibitor administered and the frequency of administration will be at the discretion of a physician.

A therapeutic advantage of using PARP inhibitors to treat cancer cells is that only very low doses are needed to have a therapeutic effect in treating cancer thereby reducing systemic build up of the inhibitors and any associated toxic effects.

Preferred features of each aspect of the invention are as for each of the other aspects *mutatis mutandis*.

The present invention will now be described by way of example only with reference to the accompanying figures, wherein:

Figure 1 is a graph showing cell survival in the presence of PARP inhibitor AG14361 in wt V79 cells, BRCA2 deficient VC-8 cells and VC-8 cells complimented with functional BRCA2 gene (VC-8#13, VC-8+B2);

Figure 2 is the human cDNA sequence of PARP-1;

Figure 3 is the human cDNA sequence of PARP-2;

Figure 4 is the human cDNA sequence of PARP-3;

Figure 5 is the human gDNA sequence of Tankyrase 1;

Figure 6 is the human mRNA sequence of Tankyrase 2;

Figure 7 is the human mRNA sequence of VPARP;

EXAMPLES

BRCA2 deficient cells are hypersensitive to PARP-1 inhibition

The survival of BRCA2 deficient cells (VC8) and wild type cells (V79Z) in the presence of PARP-1 inhibitor, AG14361, was investigated. It was found that VC8 cells are very sensitive to the toxic effect of AG14361 (Figure 1). The sensitivity in VC8 cells was corrected by the introduction of a functional BRCA2 gene either on chromosome 13 (VC8#13) or on an overexpression vector (VC8+B2). This result demonstrates that the sensitivity to PARP-1 inhibitors is a direct consequence of loss of the BRCA2 function.

Table 1. Genotype and origin of cell lines used in this study.

Cell line	Genotype	Defect	Origin	Reference
AA8	wt	wt	CHO	[41]
irs1SF	*XRCC3*$^-$	XRCC3$^-$, deficient in HR	AA8	[41]
CXR3	*XRCC3*$^-$ + *hXRCC3*	wt	irs1SF	[41]
V79-4	wt	wt	V79	[40]
irs1	*XRCC2*$^-$	XRCC2$^-$, deficient in HR	V79-4	[40]
irs1X2.2	*XRCC2*$^-$ + *hXRCC2*	wt	irs1	[40]
V79-Z	*wt*	wt	V79	[42]
VC8	*BRCA2*$^-$	BRCA2$^-$, deficient in HR	V79-Z	[42]
VC8#13	*BRCA2*$^-$ +*hBRCA2*	wt	VC8	[42]
VC8+B2	*BRCA2*$^-$ +*hBRCA2*	wt	VC8	[42]

Materials and Methods

Cytotoxicity of BRCA2 cells to PARP inhibitors

Cell culture

The irs1, irs1X2.1 and V79-4 cell lines were a donation from John Thacker [40] and the AA8, irs1SF and CXR3 cell lines were provided by Larry Thompson [41].

The VC-8, VC-8+B2, VC-8#13 were a gift from Malgorzata Zdzienicka [42]. All cell lines in this study were grown in Dulbecco's modified Eagle's Medium (DMEM) with 10% Foetalbovine serum and penicillin (100 U/ml) and streptomycin sulphate (100 μg/mL) at 37°C under an atmosphere containing 5% CO_2.

Toxicity assay - clonogenic survival assay

Exponentially growing cells in 6-well plates were exposed to AG14361 in 1% DMSO or 1% DMSO alone in medium for 24 hours.

The cells were harvested by trypsinisation, counted and seeded at varying densities in 10 cm dishes in fresh medium in the absence of drug for colony formation.

7-10 days later the dishes were fixed with methanol:acetic acid 3:1 and stained with 0.4% crystal violet.

Colonies were counted and the survival relative to 1%DMSO control treated cells calculated.

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CLAIMS

1. Use of the poly(ADP-ribose) polymerase (PARP) inhibitor, AG14361, in the manufacture of a medicament for the treatment of diseases caused by a defect in a gene that mediates homologous recombination (HR).

2. The use as claimed in claim 1 wherein the defect is a mutation in a gene encoding a protein involved in HR.

3. The use as claimed in claim 1 wherein the defect is the absence of a gene encoding a protein involved in HR.

4. The use as claimed in claim 1 wherein the defect is in the expression of a gene encoding a protein involved in HR.

5. The use as claimed in any preceding claim wherein the gene that mediates HR is selected from the group consisting of XRCC1, ADPRT (PARP-1), ADPRTL2 (PARP-2), CTPS, RPA, RPA1, RPA2, RPA3, XPD, ERCC1, XPF, MMS19, RAD51, RAD51B, RAD51C, RAD51D, DMC1, XRCC2, XRCC3, BRCA1, BRCA2, RAD52, RAD54, RAD50, MRE11, NBS1, WRN, BLM, Ku70, Ku80, ATM, ATR, chk1, chk2, FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, RAD1 and RAD9.

6. The use as claimed in any preceding claim in the treatment of cancer.

7. The use as claimed in claim 6 wherein the cancer is selected from the group consisting of lung, colon, pancreatic, gastric, ovarian, cervical, breast and prostate cancer.

8. The use as claimed in claim 6 or 7 wherein the cancer is in a human.

9. The use as claimed in any of claims 6 to 8 wherein the cancer is gene-linked hereditary cancer.

10. The use as claimed in claim 9 wherein the cancer is breast cancer.

11. The use as claimed in any of claims 6 to 10 wherein the cancer cells to be treated are defective in BRCA1 expression.

12. The use as claimed in any of claims 6 to 10 wherein the cancer cells to be treated are defective in BRCA2 expression.

13. The use as claimed in claim 11 or 12 wherein the cancer cells are partially deficient in BRCA1 and/or BRCA2 expression.

14. The use as claimed in claim 11 or 12 wherein the cancer cells are totally deficient in BRCA1 and/or BRCA2 expression.

15. The use as claimed in any preceding claim wherein the gene that mediates HR is a tumour suppressor gene.

16. The use as claimed in claim 15 wherein the tumour suppressor gene is BRCA1.

17. The use as claimed in claim 15 wherein the tumour suppressor gene is BRCA2

18. Use of the PARP inhibitor, AG14361, in the manufacture of a medicament for inducing apoptosis in HR defective cells.

19. The use as claimed in claim 18 wherein the HR defective cells are cancer cells.

20. The use as claimed in claim 19 wherein the cancer cells defective in HR are partially deficient in HR.

21. The use as claimed in claim 19 wherein the cancer cells defective in HR are totally deficient in HR.

22. A method of treatment of a disease in a mammal, including human, which is caused by a defect in a gene that mediates homologous recombination, which method

comprises administering to the mammal a therapeutically effective amount of the PARP inhibitor, AG14361.

23. A method of inducing apoptosis in HR defective cells in a mammal which method comprises administering to the mammal a therapeutically effective amount of the PARP inhibitor, AG14361.

Figure 1.

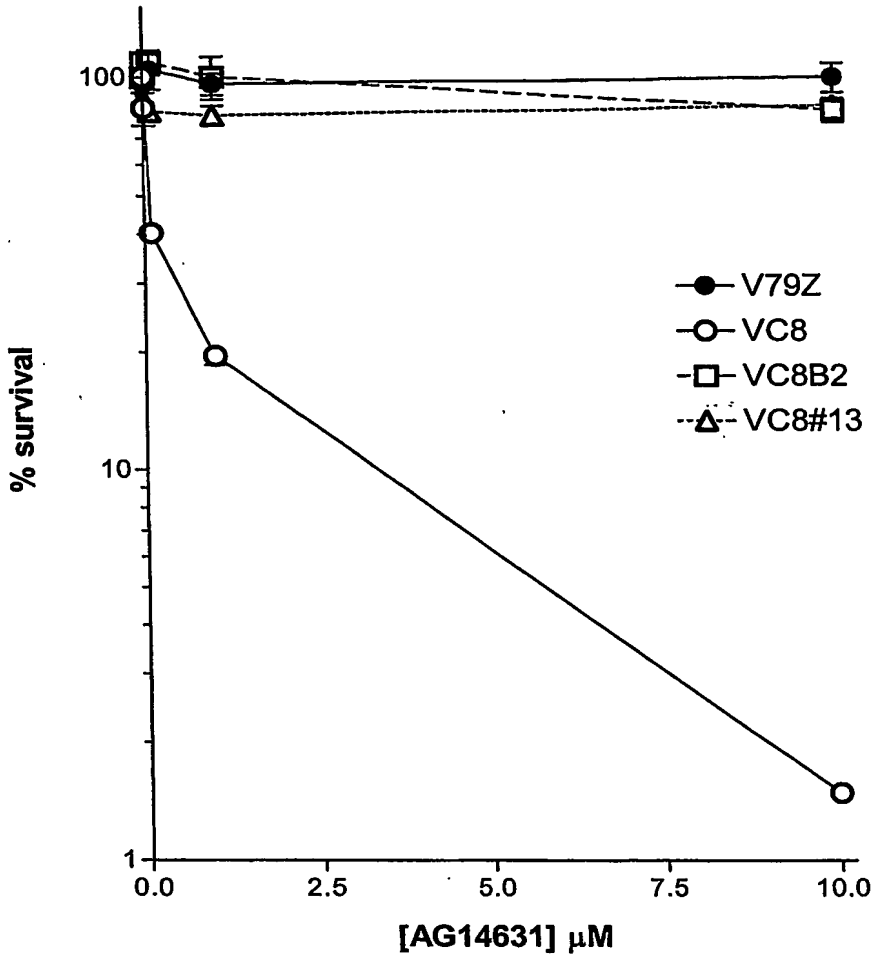
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FIGURE 2

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Figure 3

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Figure 4

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Figure 5

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Figure 6

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Figure 7

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